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=> s anti-VEGFR1
L1 28 ANTI-VEGFR1

=> s l1 and anti-VEGFR2
L2 12 L1 AND ANTI-VEGFR2

=> s l2 and pd<20010626
2 FILES SEARCHED...
L3 0 L2 AND PD<20010626

=> s l1 and pd<20010626
2 FILES SEARCHED...
L4 1 L1 AND PD<20010626

=> d l4 cbib abs

L4 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
2001:65811 Document No.: PREV200100065811. Sema3A inhibits cell migration via neuropilin and a tyrosine-kinase activity related to vascular endothelial growth factor receptor 1 (VEGFR1). Bagnard, D. [Reprint author]; Vaillant, C.; Akaoka, H.; Belin, M. F.; Puschel, A. W.; Bolz, J.; Thomasset, N.. Lyon, France. Society for Neuroscience Abstracts, **(2000)** Vol. 26, No. 1-2, pp. Abstract No.-25.7. print.
Meeting Info.: 30th Annual Meeting of the Society of Neuroscience. New Orleans, LA, USA. November 04-09, 2000. Society for Neuroscience.
ISSN: 0190-5295. Language: English.

AB We have previously reported that Sema3A acts as a repulsive guidance cue for migrating primitive neuroectodermal tumor cells (Dev cell line) (Society Neurosci Abstract Vol.25, 2035, 1999). Here, we further analyzed the

cellular mechanism involved in the transduction of Sema3A signaling. We found that Dev cells express both Neuropilin-1, the ligand-binding sub-unit of Sema3A receptor, and VEGFR1 (also called Flt-1), one of the receptor for Vascular Endothelial Growth Factor (VEGF). Using a stripe assay, we demonstrated that Dev cells avoided territories containing Sema3A. This repulsive effect was blocked both by addition of an anti-Neuropilin1 antibody and VEGF isoforms (VEGF121, VEGF165). Moreover, presence of inhibitor of tyrosine-kinase activity (genistein) also blocked Sema3A-mediated repulsion. Strikingly, inactivation of VEGFR1 using both antibody (**anti-VEGFR1**) and anti-sense targeting suppressed inhibition of cell migration in Sema3A containing substrates. It has been shown that Neuropilin requires additional co-receptor to transduce Sema3A signaling (Renzi et al., J. Neuroscience 1999; 19:7870-7880). Members of the Plexin family have been demonstrated to play such a role (Takahashi et al., 1999; Cell 99:59-69). The results presented here, suggest that VEGFR1 has a similar function and participates to Sema3A-mediated repulsion via its tyrosine-kinase activity. Thus, Sema3A signaling is mediated through complex receptor associating receptors for chemotropic signals (Neuropilin-1) to receptor of angiogenic factor (VEGFR1).

=> s anti-Flt1
L5 17 ANTI-FLT1

=> s l5 and anti-KDR
L6 1 L5 AND ANTI-KDR

=> d l6 cbib abs

L6 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
2004:167842 Document No.: PREV200400161928. Co-expression and stimulatory function of Flt1 and KDR receptors in hematopoietic stem cells. Botta, Rosanna [Reprint Author]; Pelosi, Elvira; Colonna, Lucrezia [Reprint Author]; Coppola, Simona; Calabro, Luana; Marziali, Giovanna; Perrillo, Alessandro; Valtieri, Mauro [Reprint Author]; Peschle, Cesare [Reprint Author]. Kimmel Cancer Center, T. Jefferson University, Philadelphia, PA, USA. Blood, (November 16 2003) Vol. 102, No. 11, pp. 143b. print. Meeting Info.: 45th Annual Meeting of the American Society of Hematology. San Diego, CA, USA. December 06-09, 2003. American Society of Hematology. CODEN: BLOOAW. ISSN: 0006-4971. Language: English.

AB Previous studies in humans and mice reported that VEGF receptor 2 (VEGFR2, also named KDR/Flk1) plays a functional role in hematopoietic stem cells (HSCs) (Ziegler et al, Science, 1999, Gerber et al, Nature, 2002), primitive endothelial precursors (Peichev et al, Blood, 2000) and hemangioblasts (Pelosi et al, Blood, 2002). A role for VEGFR1/Flt1 in HSC activity has also been reported (Gerber et al. Nature, 2002; Hattori et al, Nat. Med., 2002). However, several aspects of these studies remain uncertain; more important, it is not established whether the expression and function of KDR and Flt1 at HSC level is interrelated or not. Our observations in human cord blood (CB) CD34+ cells indicate that Flt1 and KDR, both expressed in a small cell subfraction (0.5-2% ot total CD34+ cells), are mostly co-expressed on the same cell subset, as evaluated by RT-PCR of Flt1 and KDR mRNA in CD34+Flt1+ vs Flt1- sorted cells and four colour flow cytometry including **anti-Flt1** and **anti-KDR** MoAb labelling of unseparated CD34+ cells. The specificity of the MoAbs for Flt1 (obtained from Reliatech, Germany; also kindly provided by Dr. M. Shibuya) and KDR (obtained from Reliatech and R&D, USA) was validated by experiments on Flt1 or KDR gene transduced cell lines. In NOD-SCID mice transplanted with CB CD34+ cells, treatment with human VEGF (10 mg i.p./day/3 days/wk/2 wks) markedly potentiates the HSC multilineage engraftment: importantly, an equivalent stimulatory effect of

multi-lineage engraftment was obtained with VEGFA (ligand for both Flt1 and Flk1), VEGFB/PlGF (ligand for Flt1) and VEGFE (ligand for Flk1, which was obtained from Reliatech; also kindly provided by Dr. K. Alitalo). The repopulating HSC activity is enriched in both CD34+KDR+ and CD34+Flt1+ subtractions, as reported; interestingly, the engraftment of CD34+KDR+ cells is potentiated by co-injection of a large number of CD34+KDR- cells releasing VEGFA. Finally, clonogenic assays indicate that both CD34+KDR+ and CD34+Flt1+ subfractions are depleted of late hematopoietic progenitor cells (HPCs), whereas they are enriched for primitive HPCs with limited self-renewal capacity (high proliferative potential colony forming cells, HPP-CFCs). Altogether our results indicate that Flt1 and KDR, essentially co-expressed on a small CD34+ cell subset enriched for primitive hematopoietic cells, stimulate the activity of HSC/primitive HPCs: these two receptors may mediate a redundant mechanism for pivotal control of HSC function via an autocrine/paracrine VEGF loop. Similarly, Flt1 and KDR functionally cooperate to stimulate megakaryocytic differentiation/maturation via an autocrine VEGF loop.

```
=> s l5 and pd<20010626
   2 FILES SEARCHED...
L7            0 L5 AND PD<20010626

=> s VEGFR
L8        16138 VEGFR

=> s l8 and antibod?
L9         3188 L8 AND ANTIBOD?

=> s l9 and combination
L10         525 L9 AND COMBINATION

=> s l10 and KDR
L11          87 L10 AND KDR

=> s l11 and Flt-1
L12          39 L11 AND FLT-1

=> s l12 and pd<20010626
   2 FILES SEARCHED...
L13           5 L12 AND PD<20010626

=> dup remove l13
PROCESSING COMPLETED FOR L13
L14           2 DUP REMOVE L13 (3 DUPLICATES REMOVED)

=> d l14 1-2 cbib abs

L14  ANSWER 1 OF 2     MEDLINE on STN                  DUPLICATE 1
2002095529.    PubMed ID: 11824379.   Involvement of VEGFR-2 (
     kdr/flk-1) but not VEGFR-1 (flt-1)
     in VEGF-A and VEGF-C-induced tube formation by human microvascular
     endothelial cells in fibrin matrices in vitro. Koolwijk P; Peters E; van
     der Vecht B; Hornig C; Weich H A; Alitalo K; Hicklin D J; Wu Y; Witte L;
     van Hinsbergh V W. (Gaubius Laboratory TNO-PG, Zernikedreef 9, 2333 CK
     Leiden, The Netherlands.. p.koolwijk@pg.tno.nl) . Angiogenesis,
     (2001) Vol. 4, No. 1, pp. 53-60.  Journal code: 9814575. ISSN:
     0969-6970. Pub. country: Netherlands. Language: English.
AB   Different forms of vascular endothelial growth factor (VEGF) and their
     cellular receptors (VEGFR) are associated with angiogenesis, as
     demonstrated by the lethality of VEGF-A, VEGFR-1 or
     VEGFR-2 knockout mice.  Here we have used an in vitro angiogenesis
```

model, consisting of human microvascular endothelial cells (hMVEC) cultured on three-dimensional (3D) fibrin matrices to investigate the roles of **VEGFR**-1 and **VEGFR**-2 in the process of VEGF-A and VEGF-C-induced tube formation. Soluble **VEGFR**-1 completely inhibited the tube formation induced by the **combination** of VEGF-A and TNF alpha (VEGF-A/TNF alpha). This inhibition was not observed when tube formation was induced by VEGF-C/TNF alpha or bFGF/TNF alpha. Blocking monoclonal **antibodies** specific for **VEGFR**-2, but not **antibodies** specifically blocking **VEGFR**-1, were able to inhibit the VEGF-A/TNF alpha-induced as well as the VEGF-C/TNF alpha-induced tube formation in vitro. PlGF-2, which interacts only with **VEGFR**-1, neither induced tube formation in **combination** with TNF alpha, nor inhibited or stimulated by itself the VEGF-A/TNF alpha-induced tube formation in vitro. These data indicate that VEGF-A or VEGF-C activation of the **VEGFR**-2, and not of **VEGFR**-1, is involved in the formation of capillary-like tubular structures of hMVEC in 3D fibrin matrices used as a model of repair-associated or pathological angiogenesis in vitro.

L14 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2009 ACS on STN
1998:463554 Document No. 129:187820 Original Reference No. 129:38133a,38136a
Increased microvascular density and enhanced leukocyte rolling and adhesion in the skin of VEGF transgenic mice. Detmar, Michael; Brown, Lawrence F.; Schon, Michael P.; Elicker, Brett M.; Velasco, Paula; Richard, Lisa; Fukumura, Dai; Monsky, Wayne; Claffey, Kevin P.; Jain, Rakesh K. (Department of Pathology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA). Journal of Investigative Dermatology, 111(1), 1-6 (English) **1998**. CODEN: JIDEAE. ISSN: 0022-202X. Publisher: Blackwell Science, Inc..

AB Vascular endothelial growth factor (VEGF) has been implicated in the pathol. angiogenesis observed in psoriasis and other chronic inflammatory skin diseases that are characterized by enhanced expression of VEGF by epidermal keratinocytes and of VEGF receptors by tortuous microvessels in the upper dermis. To investigate the functional importance of chronic VEGF overexpression in vivo, we used a keratin 14 promoter expression cassette containing the gene for murine VEGF164 to selectively target VEGF expression to basal epidermal keratinocytes in transgenic mice. These mice demonstrated an increased d. of tortuous cutaneous blood capillaries with elevated expression levels of the high affinity VEGF receptors, **VEGFR**-1 and **VEGFR**-2, most prominently during the neonatal period. In contrast, no abnormalities of lymphatic vessels were detected. In addition, the number of mast cells in the upper dermis was significantly increased in transgenic skin. Intravital fluorescence microscopy revealed highly increased leukocyte rolling and adhesion in postcapillary skin venules that were both inhibited after injection of blocking **antibodies** against E- and P-selectin. Combined blocking **antibodies** against intercellular adhesion mol.-1 and lymphocyte function-associated antigen-1 were without effect, whereas an anti-vascular cell adhesion mol.-1/ VLA-4 **antibody combination** almost completely normalized the enhanced leukocyte adhesion in transgenic mice. This study reveals VEGF as a growth factor specific for blood vessels, but not lymphatic vessels, and demonstrates that chronic orthotopic overexpression of VEGF in the epidermis is sufficient to induce cardinal features of chronic skin inflammation, providing a mol. link between angiogenesis, mast cell accumulation, and leukocyte recruitment to sites of inflammation.

=> s scFvplC11
L15 0 SCFVPLC11

=> s diabody

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L16       1290 DIABODY

=> s l16 and VEGFR
L17         15 L16 AND VEGFR

=> dup remove l17
PROCESSING COMPLETED FOR L17
L18          11 DUP REMOVE L17 (4 DUPLICATES REMOVED)

=> s l18 and pd<20010626
   2 FILES SEARCHED...
L19          1 L18 AND PD<20010626

=> d l19 cbib abs

L19  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2009 ACS on STN
2001:743201   Document No. 136:36283  Complete inhibition of vascular
     endothelial growth factor (VEGF) activities with a bifunctional
     diabody directed against both VEGF kinase receptors, fms-like
     tyrosine kinase receptor and kinase insert domain-containing receptor.
     Lu, Dan; Jimenez, Xenia; Zhang, Haifan; Wu, Yan; Bohlen, Peter; Witte,
     Larry; Zhu, Zhenping (Departments of Molecular and Cell Biology, ImClone
     Systems Inc., New York, NY, 10014, USA).  Cancer Research, 61(19),
     7002-7008 (English) 2001.  CODEN: CNREA8.  ISSN: 0008-5472.
     Publisher: American Association for Cancer Research.
AB   Vascular endothelial growth factor (VEGF) binds to and mediates its
     activity mainly through two tyrosine kinase receptors, VEGF receptor 1 [or
     fms-like tyrosine kinase receptor (Flt-1)] and VEGF receptor 2 [or kinase
     insert domain-containing receptor (KDR)].  Numerous studies have shown that
     overexpression of VEGF and its receptor plays an important role in
     tumor-associated angiogenesis and hence in both tumor growth and metastasis.
     We demonstrated previously that antagonistic antibodies to KDR
     specifically inhibited VEGF-stimulated receptor activation, cell
     migration, and endothelial cell mitogenesis.  Here we constructed a
     recombinant bifunctional diabody that is capable of blocking
     both Flt-1 and KDR from binding to their ligands, including VEGF and
     placenta growth factor (PIGF).  The diabody was expressed in
     Escherichia coli and purified by single-step affinity chromatog.  The
     diabody retained the capacity to bind both KDR and Flt-1 and
     effectively blocked interaction between KDR and VEGF, Flt-1 and VEGF, and
     Flt-1 and PIGF.  Furthermore, the diabody is a stronger
     inhibitor than its parent antibodies to VEGF-stimulated mitogenesis of
     human endothelial cells, as well as both VEGF- and PIGF-induced migration
     of human leukemia cells.  Our results suggest that dual receptor blockade
     with the bifunctional diabody may prove to be a more efficient
     approach in inhibiting VEGF-stimulated angiogenesis.


=> s bispecific antibod?
L20       5253 BISPECIFIC ANTIBOD?

=> s l20 and VEGFR
L21         21 L20 AND VEGFR

=> s l21 and pd<20010626
   2 FILES SEARCHED...
L22          0 L21 AND PD<20010626

=> s antibod?
L23    3263781 ANTIBOD?

=> s l23 and KDR
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L24       1664 L23 AND KDR

=> s l24 and Flt1
L25        112 L24 AND FLT1

=> s l25 and combination
L26          8 L25 AND COMBINATION

=> s l26 and pd<20010626
   2 FILES SEARCHED...
L27          1 L26 AND PD<20010626

=> d l27 cbib abs

L27  ANSWER 1 OF 1  CAPLUS  COPYRIGHT 2009 ACS on STN
1999:753115   Document No. 132:9411  Therapeutics containing inhibitors for
     signal transduction mediated by the vascular endothelial growth factor
     receptors.  Shitara, Kenya; Sato, Yasufumi (Kyowa Hakko Kogyo Co., Ltd.,
     Japan).  PCT Int. Appl. WO 9959636 A1 19991125, 111 pp.
     DESIGNATED STATES: W:  AU, BG, BR, CA, CN, CZ, HU, ID, IL, IN, JP, KR, MX,
     NO, NZ, PL, RO, SG, SI, SK, UA, US, VN, ZA, AM, AZ, BY, KG, KZ, MD, RU,
     TJ, TM; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,
     NL, PT, SE.  (Japanese).  CODEN: PIXXD2.  APPLICATION: WO 1999-JP2660
     19990520.  PRIORITY: JP 1998-138999 19980520.
AB   Described is a therapeutic composition against solid tumors, rheumatoid
     arthritis, diabetic retinopathy, premature retinopathy, psoriasis, etc.,
     comprising a combination of substances inhibiting the signal
     transduction mediated by human VEGF receptor Flt-1 or KDR.  The
     Flt-1-mediated signal transduction may be inhibited by a monoclonal
     antibody to Flt-1, a Flt-1 tyrosine kinase inhibitor, and a p38
     inhibitor.  The KDR-mediated signal transduction may be
     inhibited by a monoclonal antibody to KDR, a
     KDR tyrosine kinase inhibitor, and an ERK inhibitor.


=> s (zhu z?/au)
L28      25354 (ZHU Z?/AU)

=> s l28 and antibod?
L29       1324 L28 AND ANTIBOD?

=> s l29 and KDR
L30        110 L29 AND KDR

=> s l30 and Flt-1
L31         16 L30 AND FLT-1

=> dup remove l31
PROCESSING COMPLETED FOR L31
L32          8 DUP REMOVE L31 (8 DUPLICATES REMOVED)

=> d l32 1-8 cbib abs

L32  ANSWER 1 OF 8  CAPLUS  COPYRIGHT 2009 ACS on STN
2006:167761   Document No. 144:252605  Novel tetravalent bispecific
     antibodies specific to receptor tyrosine kinase for cancer
     diagnosis and therapy.  Zhu, Zhenping (Imclone Systems
     Incorporated, USA).  PCT Int. Appl. WO 2006020258 A2 20060223, 106 pp.
     DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY,
     BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB,
     GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC,
     LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO,
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NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US25472 20050718. PRIORITY: US 2004-588688P 20040717.

AB The invention is directed to novel tetravalent **antibodies**, which are preferably bispecific. The tetravalent bispecific **antibodies** have antigen-binding sites for epitopes of same or different receptor tyrosine kinase selected from VEGFR-1, VEGFR-2, VEGFR-3, **KDR**, **FLT**-1, FLT-4, EGFR, HER2, IGFR, IGF-1R, RON, FGFR, PDGFR, PDGFRα, NGFR, Tekv or Tie2. The tetravalent bispecific **antibodies** can be efficiently expressed in prokaryotic and eukaryotic cells, and are useful in therapeutic and diagnostic methods. The invention further describes administration of the **antibodies** , either alone or in combination with antiangiogenic or anti-neoplastic drugs to inhibit tumor growth and/or angiogenesis.

L32 ANSWER 2 OF 8 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

2003:373297 The Genuine Article (R) Number: 672EE. Vascular endothelial growth factor (VEGF) receptor-2 signaling mediates VEGF-C-Delta N Delta C- and VEGF-A-induced angiogenesis in vitro.
Pepper M S (Reprint). Ctr Med Univ Geneva, Dept Cell Biol & Morphol, 1 Rue Michel Servet, CH-1211 Geneva 4, Switzerland (Reprint). Tille J C; Wang X Y; Lipson K E; McMahon G; Ferrara N; **Zhu Z P**; Hicklin D J; Sleeman J P; Eriksson U; Alitalo K. Ctr Med Univ Geneva, Dept Cell Biol & Morphol, CH-1211 Geneva 4, Switzerland; SUGEN Inc, San Francisco, CA USA; Genentech Inc, San Francisco, CA 94080 USA; ImClone Syst Inc, New York, NY USA; Forschungszentrum Karlsruhe, Inst Toxicol & Genet, D-76021 Karlsruhe, Germany; Ludwig Inst Canc Res, S-10401 Stockholm, Sweden; Univ Helsinki, Biomedicum, Mol Canc Biol Lab, Helsinki, Finland.
EXPERIMENTAL CELL RESEARCH (1 MAY 2003) Vol. 285, No. 2, pp. 286-298. ISSN: 0014-4827. Publisher: ACADEMIC PRESS INC ELSEVIER SCIENCE, 525 B ST, STE 1900, SAN DIEGO, CA 92101-4495 USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Angiogenesis and lymphangiogenesis are regulated by members of the vascular endothelial growth factor (VEGF) family of cytokines, which mediate their effects via tyrosine kinase VEGF receptors -1, -2, and -3. We have used wild-type and mutant forms of VEGFs -A, -B, and -C, a pan-VEGFR tyrosine kinase inhibitor (SU5416) as well as neutralizing anti-VEGFR-2 **antibodies**, to determine which VEGF receptor(s) are required for bovine endothelial cell invasion and tube formation in vitro. This was compared to the ability of these cytokines to induce expression of members of the plasminogen activator (PA)-plasmin system. We found that cytokines which bind VEGFR-2 (human VEGF-A, human VFM23A, human VEGF-C-DeltaNDeltaC, and rat VEGF-C-152) induced invasion, tube formation, urokinase-type-PA, tissue-type-PA, and PA inhibitor-1, invasion and tube formation as well as signaling via the MAP kinase pathway were efficiently blocked by SU5416 and anti-VEGFR-2 **antibodies**. In contrast, cytokines and mutants which exclusively bind VEGFR-1 (human VFM17 and human VEGF-B) had no effect on invasion and tube formation or on the regulation of gene expression. We were unable to identify cytokines which selectively stimulate bovine VEGFR-3 in our system. Taken together, these findings point to the central role of VEGFR-2 in the angiogenic signaling pathways induced by VEGF-C-DeltaNDeltaC and VEGF-A. (C) 2003 Elsevier Science (USA). All rights reserved.

L32 ANSWER 3 OF 8 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

2003:805061 The Genuine Article (R) Number: 722ND. Di-diabody: a novel tetravalent bispecific **antibody** molecule by design.

Zhu Z P (Reprint). ImClone Syst Inc, Dept Antibody Technol, 180 Varick St, New York, NY 10014 USA (Reprint). Lu D; Jimenez X; Zhang H F; Atkins A; Brennan L; Balderes P; Bohlen P; Witte L. ImClone Syst Inc, Dept Antibody Technol, New York, NY 10014 USA; ImClone Syst Inc, Dept Mol & Cell Biol, New York, NY 10014 USA; ImClone Syst Inc, Dept Prot Chem, New York, NY 10014 USA; ImClone Syst Inc, Res Dept, New York, NY 10014 USA. JOURNAL OF IMMUNOLOGICAL METHODS (AUG 2003) Vol. 279, No. 1-2, pp. 219-232. ISSN: 0022-1759. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The clinical development of bispecific **antibodies** (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. In recent years, a variety of recombinant methods have been developed for efficient production of BsAb, both as **antibody** fragments and as full-length IgG-like molecules. These recombinant **antibody** molecules possess dual antigen-binding capability with, in most cases, monovalency to each of their target antigens. Here, we describe an efficient approach for the production of a novel tetravalent BsAb, with two antigen-binding sites to each of its target antigens, by genetically fusing a bispecific/divalent diabody to, via the hinge region, the N-terminus of the CH3 domain of an IgG. The novel BsAb, which we termed "di-diabody", represents a tetravalent diabody dimer resulting from dimerization between the hinge region and the CH3 domains. A di-diabody was constructed using two **antibodies** directed against the two tyrosine kinase receptors of vascular endothelial growth factor, expressed both in a single Escherichia coli host and in mammalian cells, and purified to homogeneity by a one-step affinity chromatography. Compared to the bispecific/divalent diabody, the tetravalent di-diabody binds more efficiently to both of its target antigens and is more efficacious in blocking ligand binding to the receptors. The di-diabody retained good antigen-binding activity after incubation at 37 degreesC in mouse serum for 72 h, demonstrating good product stability. Finally, expression of the di-diabody in mammalian cells yielded higher level of production and better **antibody** activity. This design and expression for BsAb fragments should be applicable to any pair of antigen specificities. (C) 2003 Elsevier B.V. All rights reserved.

L32 ANSWER 4 OF 8 MEDLINE on STN DUPLICATE 1
2002050877. PubMed ID: 11774295. Selection of high affinity human neutralizing **antibodies** to VEGFR2 from a large **antibody** phage display library for antiangiogenesis therapy. Lu Dan; Jimenez Xenia; Zhang Haifan; Bohlen Peter; Witte Larry; **Zhu Zhenping**. (Department of Molecular and Cell Biology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.) International journal of cancer. Journal international du cancer, (2002 Jan 20) Vol. 97, No. 3, pp. 393-9. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Compelling evidence suggests that vascular endothelial growth factor (VEGF) and its receptors play an important role in angiogenesis associated with tumor growth and metastasis. VEGF exerts its biologic activities through 2 transmembrane tyrosine kinase receptors: the fms-like tyrosine kinase receptor (**Flt-1**, or VEGFR1) and kinase insert domain-containing receptor (**KDR** or VEGFR2). We have previously produced a panel of **antibodies** directed against **KDR** from mice immunized with the recombinant form receptor. These **antibodies** efficiently neutralized VEGF-induced **KDR** activation and mitogenesis of human umbilical vascular endothelial cells (HUVEC). Murine **antibodies**, however, may not be suitable candidates for human therapy because of their propensity to elicit human anti-mouse **antibody** response. Here we isolated several high-affinity human Fab **antibody** fragments directed against

KDR from an **antibody** phage display library constructed from the pooled B lymphocytes of nonimmunized healthy human donors. These human Fab fragments bind specifically to **KDR** with nanomolar affinity and block **KDR**/VEGF interaction with IC(50) of approximately 2-20 nM. Further, they effectively inhibit VEGF-stimulated mitogenesis of HUVEC and migration of human leukemia cells. Epitope mapping studies demonstrated that all neutralizing human **antibodies** bound the epitope(s) located within the first 3 N-terminal immunoglobulin-like domains of **KDR**, the same region that encompasses the binding site of VEGF. Our results suggest that these human anti-**KDR antibodies** may have potential application in the treatment of cancer and other diseases in which pathologic angiogenesis occurs.
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L32 ANSWER 5 OF 8 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN DUPLICATE 2
2002:557780 Document No.: PREV200200557780. Fab-scFv fusion protein: An efficient approach to production of bispecific **antibody** fragments. Lu, Dan; Jimenez, Xenia; Zhang, Haifan; Bohlen, Peter; Witte, Larry; **Zhu, Zhenping** [Reprint author]. Department of Antibody Technology, ImClone Systems Incorporated, 180 Varick Street, New York, NY, 10014, USA. Zhenping@imclone.com. Journal of Immunological Methods, (15 September, 2002) Vol. 267, No. 2, pp. 213-226. print.
CODEN: JIMMBG. ISSN: 0022-1759. Language: English.
AB The clinical development of bispecific **antibodies** (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. Here, we describe an efficient approach for the production of a novel bispecific **antibody** fragment by genetically fusing a single-chain Fv (scFv) to the C-terminus of either the light chain or the heavy chain of a Fab fragment of different antigen-binding specificity. The bispecific Fab-scFv fragments were expressed in a single Escherichia coli host and purified to homogeneity by a one-step affinity chromatography. Two different versions of the bispecific Fab-scFv fragments were constructed using two **antibodies** directed against the two tyrosine kinase receptors of vascular endothelial growth factor. These bispecific **antibody** fragments not only retained the antigen-binding capacity of each of the parent **antibodies**, but also are capable of binding to both targets simultaneously as demonstrated by a cross-linking ELISA. Further, the bispecific **antibodies** were comparable to their parent **antibodies** in their potency in blocking ligand binding to the receptors and in inhibiting ligand-induced biological activities. This design for BsAb fragments should be applicable to any pair of antigen specificities.

L32 ANSWER 6 OF 8 MEDLINE on STN DUPLICATE 3
2001538782. PubMed ID: 11585724. Complete inhibition of vascular endothelial growth factor (VEGF) activities with a bifunctional diabody directed against both VEGF kinase receptors, fms-like tyrosine kinase receptor and kinase insert domain-containing receptor. Lu D; Jimenez X; Zhang H; Wu Y; Bohlen P; Witte L; **Zhu Z.** (Department of Molecular and Cell Biology, ImClone Systems Inc., New York, New York 10014, USA.) Cancer research, (2001 Oct 1) Vol. 61, No. 19, pp. 7002-8. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States.
Language: English.
AB Vascular endothelial growth factor (VEGF) binds to and mediates its activity mainly through two tyrosine kinase receptors, VEGF receptor 1 [or fms-like tyrosine kinase receptor (**Flt-1**)] and VEGF receptor 2 [or kinase insert domain-containing receptor (**KDR**)]. Numerous studies have shown that overexpression of VEGF and its receptor plays an important role in tumor-associated angiogenesis and hence in both

tumor growth and metastasis. We demonstrated previously that antagonistic **antibodies** to **KDR** specifically inhibited VEGF-stimulated receptor activation, cell migration, and endothelial cell mitogenesis. Here we constructed a recombinant bifunctional diabody that is capable of blocking both **Flt-1** and **KDR** from binding to their ligands, including VEGF and placenta growth factor (PlGF). The diabody was expressed in Escherichia coli and purified by single-step affinity chromatography. The diabody retained the capacity to bind both **KDR** and **Flt-1** and effectively blocked interaction between **KDR** and VEGF, **Flt-1** and VEGF, and **Flt-1** and PlGF. Furthermore, the diabody is a stronger inhibitor than its parent **antibodies** to VEGF-stimulated mitogenesis of human endothelial cells, as well as both VEGF- and PlGF-induced migration of human leukemia cells. Taken together, our results suggest that dual receptor blockade with the bifunctional diabody may prove to be a more efficient approach in inhibiting VEGF-stimulated angiogenesis.

L32 ANSWER 7 OF 8 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
2002:220588 Document No.: PREV200200220588. Activation of VEGF receptor-1 (VEGFR1, **Flt-1**) expressed on hematopoietic stem cells promotes cell motility and is essential for marrow reconstitution.
Hattori, Koichi [Reprint author]; Heissig, B. [Reprint author]; Dias, S. [Reprint author]; Hicklin, D. J.; Wu, Y.; Witte, L.; **Zhu, Z.**; Lyden, D.; Hendrikx, P. J.; Visser, J. W. M.; Crystal, R. G.; Moore, M. A. S.; Rafii, S. [Reprint author]. Division of Hematology-Oncology, Cornell University Medical College, New York, NY, USA. Blood, (November 16, 2001) Vol. 98, No. 11 Part 1, pp. 710a-711a. print.
Meeting Info.: 43rd Annual Meeting of the American Society of Hematology, Part 1. Orlando, Florida, USA. December 07-11, 2001. American Society of Hematology.
CODEN: BLOOAW. ISSN: 0006-4971. Language: English.
AB Compelling evidence suggest that the angiogenic switch is associated with co-activation and recruitment of endothelial precursor cells and hematopoietic stem cells (HSC). However, the mechanism(s) whereby angiogenic factors interact with HSCs and promote hematopoiesis are not well known. In search of signaling and cellular mediators common to both hematopoietic and angiogenic cascades, we have found that functional vascular endothelial growth factor receptor-1 (VEGFR1, **Flt-1**) is expressed on a subpopulation of CD34+ human NOD/SCID repopulating cells and mouse (Sca-1+c-kit+) cells with stem cell potential. Transplantation of as low as one thousand purified mouse Sca-1+VEGFR1+ cells obtained from the bone marrow (BM) of Ly5.2 mice reconstituted hematopoiesis in lethally irradiated Ly5.1 mice. Flow cytometric analysis of the peripheral blood of mice transplanted with Sca-1+VEGFR1+Ly5.2+ cells after 150 days, demonstrated that 91+-5% of the mononuclear cells comprised of donor derived lymphoid (Ly5.2+B220+, Ly5.2+Thy-1+), myeloid (Ly5.2+Gr1+, Ly5.2+CD11b+) cells, suggesting that Sca-1+VEGFR1+ cells contain long-term repopulating cells. To define the mechanism whereby VEGFR1 may regulate hematopoiesis, we took advantage of placental derived growth factor (PlGF) which exclusively signals through VEGFR1, but not VEGFR2 (**KDR**, Flk1). We demonstrate that plasma elevation of PlGF ameliorates the extent and duration of neutropenia after myeloablative doses of chemotherapy (Carboplatin 1.2 mg+irradiation 5 Gy)-induced BM suppression, suggesting that functional VEGFR1 is expressed on HSCs. Conversely, inhibition of VEGFR1, but not VEGFR2 signaling, by neutralizing monoclonal **antibody**, blocks hematopoietic recovery after BM suppression, resulting in the demise of 40% of the treated mice, and profound delay in leukocyte recovery in the remaining surviving mice. In order to define the mechanism whereby VEGFR1 activation enhances BM recovery, we demonstrate that PlGF promotes expansion of Sca-1+ cells in S and G2/M phase of the cell cycle. This increase in cell cycling is not

due to direct effect of PlGF on cell proliferation, since activation of VEGFR1 does not influence HSC cell survival or colony expansion. We show that PlGF induces cell cycling by enhancing cell motility, through the activation of matrix metalloproteinase-9 (MMP-9) expressed in BM stromal cells. Indeed, compared to wild-type mice, PlGF failed to mobilize HSCs in MMP-9 deficient mice. We also demonstrate that PlGF through interaction with VEGFR1, induces MMP-9 production and secretion by HSCs, enhancing their migration through reconstituted collagen matrix. These data suggest that inhibition of VEGFR1 signaling blocks the motogenic (cell motility), but not mitogenic (cell proliferation), potential of HSCs, thereby impeding their entry into a permissive environment that is essential for cell cycling and mobilization. Based on these studies, we propose that angiogenic factors promote hematopoiesis through interaction with functional VEGFR1, but not VEGFR2, and are essential for reconstitution of hematopoiesis.

L32 ANSWER 8 OF 8 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN

2000:381906 The Genuine Article (R) Number: 313NZ. Identification of the residues in the extracellular region of **KDR** important for interaction with vascular endothelial growth factor and neutralizing anti-**KDR antibodies.**

Zhu Z P (Reprint). ImClone Syst Inc, Dept Mol & Cell Biol, 180 Varick St, New York, NY 10014 USA (Reprint). Lu D; Kussie P; Pytowski B; Persaud K; Bohlen P; Witte L. ImClone Syst Inc, Dept Mol & Cell Biol, New York, NY 10014 USA; ImClone Syst Inc, Dept Prot Chem, New York, NY 10014 USA; ImClone Syst Inc, Dept Res, New York, NY 10014 USA.

JOURNAL OF BIOLOGICAL CHEMISTRY (12 MAY 2000) Vol. 275, No. 19, pp. 14321-14330. ISSN: 0021-9258. Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814 USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The kinase domain receptor (**KDR**) of vascular endothelial growth factor (VEGF) is the main human receptor responsible for the angiogenic activity of VEGF. The extracellular region of **KDR** is comprised of seven immunoglobulin-like domains, of which the first three have been shown to be required for ligand binding. We have previously described **antibodies** directed against the extracellular region of **KDR**, including MAB383 and MAB664, which were shown to block the binding of VEGF to the receptor and to inhibit both VEGF-induced mitogenesis of human endothelial cells in vitro and tumor growth in vivo. Here we generated a series of **KDR** deletion mutants consisting of truncated extracellular regions and mapped out the domain(s) responsible for binding to VEGF and the neutralizing anti-**KDR antibodies**. All neutralizing **antibodies** were found to require domain 3 for efficient binding. Alanine-scanning mutagenesis of domain 3 identified two different sets of five residues, Ile(256), Asp(257), Glu(261), Leu(313) and Thr(315) and Tyr(262), Pro(263), Ser(264), Ser(265), and Lys(266), that were critical for binding to MAB383 and MAB664, respectively. Combination of alanine mutations affecting both MAB383 and MAB664 binding resulted in a variant that also lost binding to VEGF. These results suggest that the residues within this region of domain 3 are critical for VEGF binding. Our studies provide a basis for the mechanism of action of our anti-**KDR antibodies** and establish a functional foundation for the development of other classes of antagonists to the receptor.

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L34 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2009 ACS on STN
2006:167761 Document No. 144:252605 Novel tetravalent **bispecific antibodies** specific to receptor tyrosine kinase for cancer diagnosis and therapy. **Zhu, Zhenping** (Imclone Systems Incorporated, USA). PCT Int. Appl. WO 2006020258 A2 20060223, 106 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IS, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2005-US25472 20050718. PRIORITY: US 2004-588688P 20040717.

AB The invention is directed to novel tetravalent **antibodies**, which are preferably **bispecific**. The tetravalent **bispecific antibodies** have antigen-binding sites for epitopes of same or different receptor tyrosine kinase selected from VEGFR-1, VEGFR-2, VEGFR-3, **KDR**, FLT-1, FLT-4, EGFR, HER2, IGFR, IGF-1R, RON, FGFR, PDGFR, PDGFRα, NGFR, Tekv or Tie2. The tetravalent **bispecific antibodies** can be efficiently expressed in prokaryotic and eukaryotic cells, and are useful in therapeutic and diagnostic methods. The invention further describes administration of the **antibodies**, either alone or in combination with antiangiogenic or anti-neoplastic drugs to inhibit tumor growth and/or angiogenesis.

L34 ANSWER 2 OF 7 SCISEARCH COPYRIGHT (c) 2009 The Thomson Corporation on STN
2003:805061 The Genuine Article (R) Number: 722ND. Di-diabody: a novel tetravalent **bispecific antibody** molecule by design.
Zhu Z P (Reprint). ImClone Syst Inc, Dept Antibody Technol, 180 Varick St, New York, NY 10014 USA (Reprint). Lu D; Jimenez X; Zhang H F; Atkins A; Brennan L; Balderes P; Bohlen P; Witte L. ImClone Syst Inc, Dept Antibody Technol, New York, NY 10014 USA; ImClone Syst Inc, Dept Mol & Cell Biol, New York, NY 10014 USA; ImClone Syst Inc, Dept Prot Chem, New York, NY 10014 USA; ImClone Syst Inc, Res Dept, New York, NY 10014 USA. JOURNAL OF IMMUNOLOGICAL METHODS (AUG 2003) Vol. 279, No. 1-2, pp. 219-232. ISSN: 0022-1759. Publisher: ELSEVIER SCIENCE BV, PO BOX 211, 1000 AE AMSTERDAM, NETHERLANDS. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The clinical development of **bispecific antibodies** (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. In recent years, a variety of recombinant methods have been developed for efficient production of BsAb, both as **antibody** fragments and as full-length IgG-like molecules. These recombinant **antibody** molecules possess dual antigen-binding capability with, in most cases, monovalency to each of their target antigens. Here, we describe an efficient approach for the production of a novel tetravalent BsAb, with two antigen-binding sites to each of its target antigens, by genetically fusing a **bispecific**/divalent diabody to, via the hinge region, the N-terminus of the CH3 domain of an IgG. The novel BsAb, which we termed "di-diabody", represents a tetravalent diabody dimer resulting from dimerization between the hinge region and the CH3 domains. A di-diabody was constructed using two **antibodies** directed against the two tyrosine kinase receptors of vascular endothelial growth factor, expressed

both in a single Escherichia coli host and in mammalian cells, and purified to homogeneity by a one-step affinity chromatography. Compared to the **bispecific**/divalent diabody, the tetravalent di-diabody binds more efficiently to both of its target antigens and is more efficacious in blocking ligand binding to the receptors. The di-diabody retained good antigen-binding activity after incubation at 37 degreesC in mouse serum for 72 h, demonstrating good product stability. Finally, expression of the di-diabody in mammalian cells yielded higher level of production and better **antibody** activity. This design and expression for BsAb fragments should be applicable to any pair of antigen specificities. (C) 2003 Elsevier B.V. All rights reserved.

L34 ANSWER 3 OF 7 BIOSIS COPYRIGHT (c) 2009 The Thomson Corporation on STN
DUPLICATE 1
2002:557780 Document No.: PREV200205577780. Fab-scFv fusion protein: An efficient approach to production of **bispecific antibody** fragments. Lu, Dan; Jimenez, Xenia; Zhang, Haifan; Bohlen, Peter; Witte, Larry; **Zhu, Zhenping** [Reprint author]. Department of Antibody Technology, ImClone Systems Incorporated, 180 Varick Street, New York, NY, 10014, USA. Zhenping@imclone.com. Journal of Immunological Methods, (15 September, 2002) Vol. 267, No. 2, pp. 213-226. print.
CODEN: JIMMBG. ISSN: 0022-1759. Language: English.
AB The clinical development of **bispecific antibodies** (BsAb) as therapeutics has been hampered by the difficulty in preparing the materials in sufficient quantity and quality by traditional methods. Here, we describe an efficient approach for the production of a novel **bispecific antibody** fragment by genetically fusing a single-chain Fv (scFv) to the C-terminus of either the light chain or the heavy chain of a Fab fragment of different antigen-binding specificity. The **bispecific** Fab-scFv fragments were expressed in a single Escherichia coli host and purified to homogeneity by a one-step affinity chromatography. Two different versions of the **bispecific** Fab-scFv fragments were constructed using two **antibodies** directed against the two tyrosine kinase receptors of vascular endothelial growth factor. These **bispecific antibody** fragments not only retained the antigen-binding capacity of each of the parent **antibodies**, but also are capable of binding to both targets simultaneously as demonstrated by a cross-linking ELISA. Further, the **bispecific antibodies** were comparable to their parent **antibodies** in their potency in blocking ligand binding to the receptors and in inhibiting ligand-induced biological activities. This design for BsAb fragments should be applicable to any pair of antigen specificities.

L34 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2009 ACS on STN
2001:868530 Document No. 136:19113 **Bispecific** immunoglobulin-like antigen binding proteins and method of production. **Zhu, Zhenping** (Imclone Systems Incorporated, USA). PCT Int. Appl. WO 2001090192 A2 20011129, 64 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US16924 20010524. PRIORITY: US 2000-206749P 20000524.
AB The present invention is directed to **bispecific** antigen-binding protein. These **bispecific** antigen-binding proteins are optimized in their avidity for antigen(s) but maintain their ability to function as a natural **antibody**, including the ability to

activate complement mediated cytotoxicity and **antibody** dependent cellular toxicity. Natural IgG Igs are monospecific and bivalent, having two binding domains which are specific for the same epitope. By contrast, an IgG type antigen-binding protein of the invention is **bispecific** and bivalent, having a binding domain on each light chain for one epitope and a binding domain on each heavy chain specific for a second epitope. The design of the present antigen-binding proteins provides for efficient production such that substantially all of the antigen-binding proteins produced are assembled in the desired configuration.

L34 ANSWER 5 OF 7 MEDLINE on STN
2001538782. PubMed ID: 11585724. Complete inhibition of vascular endothelial growth factor (VEGF) activities with a bifunctional diabody directed against both VEGF kinase receptors, fms-like tyrosine kinase receptor and kinase insert domain-containing receptor. Lu D; Jimenez X; Zhang H; Wu Y; Bohlen P; Witte L; **Zhu Z.** (Department of Molecular and Cell Biology, ImClone Systems Inc., New York, New York 10014, USA.) Cancer research, (2001 Oct 1) Vol. 61, No. 19, pp. 7002-8. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Vascular endothelial growth factor (VEGF) binds to and mediates its activity mainly through two tyrosine kinase receptors, VEGF receptor 1 [or fms-like tyrosine kinase receptor (Flt-1)] and VEGF receptor 2 [or kinase insert domain-containing receptor (**KDR**)]. Numerous studies have shown that overexpression of VEGF and its receptor plays an important role in tumor-associated angiogenesis and hence in both tumor growth and metastasis. We demonstrated previously that antagonistic **antibodies** to **KDR** specifically inhibited VEGF-stimulated receptor activation, cell migration, and endothelial cell mitogenesis. Here we constructed a recombinant bifunctional diabody that is capable of blocking both Flt-1 and **KDR** from binding to their ligands, including VEGF and placenta growth factor (PlGF). The diabody was expressed in Escherichia coli and purified by single-step affinity chromatography. The diabody retained the capacity to bind both **KDR** and Flt-1 and effectively blocked interaction between **KDR** and VEGF, Flt-1 and VEGF, and Flt-1 and PlGF. Furthermore, the diabody is a stronger inhibitor than its parent **antibodies** to VEGF-stimulated mitogenesis of human endothelial cells, as well as both VEGF- and PlGF-induced migration of human leukemia cells. Taken together, our results suggest that dual receptor blockade with the bifunctional diabody may prove to be a more efficient approach in inhibiting VEGF-stimulated angiogenesis.

L34 ANSWER 6 OF 7 MEDLINE on STN DUPLICATE 2
2000295268. PubMed ID: 10835110. An efficient route to the production of an IgG-like **bispecific antibody**. Zuo Z; Jimenez X; Witte L; **Zhu Z.** (Department of Molecular and Cell Biology, ImClone Systems Incorporated, 180 Varick Street, New York, NY 10014, USA.) Protein engineering, (2000 May) Vol. 13, No. 5, pp. 361-7. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Production of IgG-form **bispecific antibody** (BsAb-IgG) by co-expressing two **antibodies** in transfected cells is often inefficient owing to the unwanted pairing between the component heavy and light chains. We have developed an efficient method for the production of a novel IgG-like BsAb by using the natural dimerization mechanism between IgG heavy and light chains. Two single-chain Fv (scFv) of different specificity are fused to the constant domain of human kappa chain (C(L)) and the first constant domain of human heavy chain (C(H1)), to form two polypeptides, (scFv)(1)-C(L) and (scFv)(2)-C(H1)-C(H2)-C(H3), respectively. Co-expression of the two polypeptides in mammalian cells results in the formation of a covalently linked IgG-like hetero-tetramer,

Bs(scFv)(4)-IgG, with dual specificity. Our approach yields a homogeneous **bispecific** IgG-like **antibody** product with each molecule containing four antigen binding sites, two for each of its target antigens. A Bs(scFv)(4)-IgG was prepared using two scFv **antibodies** each directed against a different epitope of a vascular endothelial growth factor receptor, the kinase insert domain-containing receptor (**KDR**). The Bs(scFv)(4)-IgG is capable of simultaneously binding to the two epitopes on the receptor. Further, the Bs(scFv)(4)-IgG also retains the antigen-binding efficacy and biological activity of its component **antibodies**.

L34 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 3
2000062952. PubMed ID: 10594363. Acquired antagonistic activity of a **bispecific** diabody directed against two different epitopes on vascular endothelial growth factor receptor 2. Lu D; Kotanides H; Jimenez X; Zhou Q; Persaud K; Bohlen P; Witte L; **Zhu Z.** (Department of Molecular and Cell Biology, ImClone Systems, 180 Varick Street, New York, NY 10014, USA.) Journal of immunological methods, (1999 Nov 19) Vol. 230, No. 1-2, pp. 159-71. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB **Bispecific antibody** (BsAb) technology has been successfully used as a means to construct novel **antibody** (Ab) molecules with increased avidity for binding, by combining two Ab or their fragments directed against different epitopes within the same antigen. Using two single chain **antibodies** (scFv) isolated from a phage display library, we have constructed a **bispecific** diabody directed against two different epitopes on the extracellular domain (ECD) of human vascular endothelial growth factor receptor 2 (VEGFR2), the kinase-insert domain-containing receptor (**KDR**). Neither of the parent scFv blocks **KDR**/VEGF interactions or inhibits VEGF-induced receptor activation. The diabody binds to **KDR** with an affinity that is 1.5- to 3-fold higher than its parent scFv, mainly due to a much slower dissociation rate (k(off)), which is approximately 17- to 26-fold slower than that of the individual scFv. In addition, the diabody binds simultaneously to, and thus cross-links, the two epitopes on the receptor(s). It is rather unexpected that the diabody effectively blocked **KDR**/VEGF interactions, and inhibited both VEGF-induced activation of the receptor and mitogenesis of human endothelial cells. Taken together, our results suggest that the diabody is most likely to exert its effect through steric hindrance and/or causing major conformational changes of the receptor. This is the first report on the construction of a **bispecific** diabody with acquired novel antagonistic activity.

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